NASA TECHNICAL TRANSLATION NASA TT F-15,421

THE SYNTHESIS OF ADENOSINE-5'-CHLORACETOPHOSPHONATE — A SPECIFIC INHIBITOR OF ACETYL-CoA-SYNTHETASE

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Translation of: "Sintez adenozin-5'-khloratsetofosfonata — spetsificheskogo ingibitora atsetil-KoA-sintetazy," Doklady Akademii nauk SSSR, Volume 213, No. 2, 1973, pp. 335-337

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D. C. 20546 MARCH 1974

STANDARD TITLE PAGE

1. Report No. NASA TT F-15,421	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle THE SYNTHESIS OF ADENOSINE-5'--CHLORACETOPHOSPHONATE – A SPECIFIC INHIBITOR OF ACETYL-CoA-SYNTHETASE		5. Report Date MARCH 1974 6. Performing Organization Code
7. Author(s) N. N. GULYAYEV AND L. A. BARANOVA		8. Performing Organization Report No. 10. Work Unit No.
9. Performing Organization Name and Address Techtran Corporation P.O. Box 729 Glen Burnie, Maryland 21061		11. Contract or Grant No. NASw-2485 13. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address NATIONAL AERONAUTICS AND SPACE ADMINISTRATION WASHINGTON, D. C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes

Translation of: "Sintez adenozin-5'-khloratsetofosfonata [sic] – spetsificheskogo ingibitora atsetil-KoA-sintetazy," Doklady Akademii nauk SSSR, Volume 213, No. 2, 1973, pp. 335-337

16. Abstract

A description for the preparation of the acetyl-CoA-synthetase inhibitor adenosine-5'-chloracetophosphonate. The processes used by the authors to obtain the compound are given. One mention is made of an *in vitro* experiment performed using a rabbit's heart. Enzymatic production was decreased in the presence of the compound.

17. Key Words (Selected by Author(s))	18. Distribution Statement
	Unclassified-Unlimited

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	76	$4.00

THE SYNTHESIS OF ADENOSINE-5'-CHLORACETOPHOSPHONATE — A SPECIFIC INHIBITOR OF ACETYL-CoA-SYNTHETASE[1]

N. N. Gulyayev and L. A. Baranova[2]

During the investigation of the mechanism of action of enzymes, specific /335* inhibitors which have high affinity for the active center and which have alkylizing groups in their composition have found wide application. The replacement of the carboxyl group with the haliodmethylketone group for derivatives of L-phenylalanine, L-lysine, L-valine and other amino acids lead to covalent blocking of the functional groups of the active center chymotrypsin [1], trypsin [2], the aminoacyl-tRNA-synthetase series, and those of other enzymes [3] by the obtained compounds.

Earlier we studied the inhibiting effect of certain acetyladenylate analogs with respect to acetyl-CoA-synthetase [4]. The most pronounced inhibiting activity with respect to the given enzyme was that of adenosine-5'-acetophosphonate, which was apparently caused by the close correspondence of the geometric parameters of the active center of the enzyme and the molecule of the inhibitor.

Taking into account the high affinity of adenosine-5'-acetophosphonate for the active center of acetyl-CoA-synthetase, it would be interesting to obtain an analogous compound, but one capable of irreversible binding with the functional groups of the enzyme. Most promising is the replacement of the acetyl group of the given compound with the chlormethylketone group, inasmuch as the latter must be spatially near the functional group of the active center which participates in transport of the acetyl residual to the SH-group of CoA during binding with the enzyme.

This study is devoted to synthesis of the previously unknown adenosine-5'--chloracetophosphonate. To obtain the 5'-chloracetophosphonate ester of adenosine, it was necessary to carry out phosphorylation of the 5'-hydroxyl

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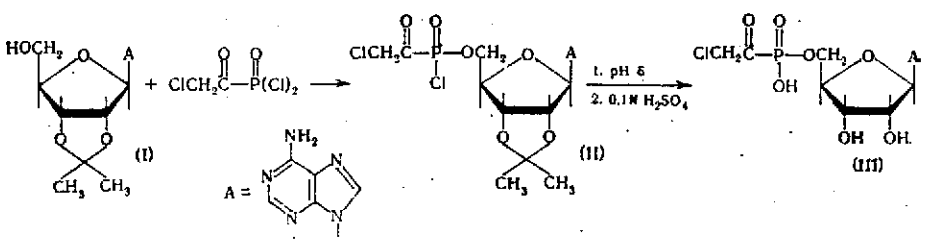
[1]Presented by Academician S. Ye. Severin 25 May 1973.
[2]M. V. Lomonosov Moscow State University.
*Numbers in the margin indicate pagination in the foreign text.

group of adenosine by the corresponding ketophosphonic acid or its derivatives. In the chemistry of nucleotides and nucleosides, many methods of obtaining 5'-phosphonate esters of nucleosides are known [4, 5], however in our case the determinative factors was obtaining the actual derivatives of chloracetophosphonic acid, inasmuch as the latter were little studied.

In the literature, there were indications of a patent character of the possibility of obtaining dialkyl esters of chloracetophosphonic acid during the reaction of chloracetylchloride with triethylphosphite [6] or with sodium diethylphosphite [7]. However, the reaction of the haloidacetylchlorides with the trialkylphosphites, as was shown by a number of authors [8], does not lead to the formation of esters of ketophosphonic acid. During the reaction of chloranhydrides of carboxylic acids with sodium diethylphosphite, ketophosphonic esters are formed as intermediate products which, however, react further with the excess amount of sodium diethylphosphite [9].

We investigated the conditions of reaction of chloracetylchloride with sodium diethylphosphite and found that when the reaction was carried out at a decreased temperature (~ -15°) and using an order of adding the reagents which provides a constant excess of chloracetylchloride in the reaction mixture, one succeeds in obtaining the diethyl ester of chloracetophosphonic acid.

Phosphorylation of the 5'-hydroxyl group of 2',3'-0-isopropylidenadenosine (I) was carried out using chloracetophosphoric acid saturated with chloranhydride; the chloracetophosphoric acid was obtained from the diethyl ester of chloracetophosphonic acid and phosphorous pentachloride. After saponification of the chloranhydride (II) formed as the result of phosphorylation and hydrolysis of the isopropylidene shield, adenosine-5'-chloracetophosphonate (III) was obtained. /336



The synthesized substance (III) proved to be homogeneous during electrophoresis and paper chromatography, and its infrared spectrum was found to contain an intensive band of absorption characteristic for the C = O group (1724 cm^{-1}).

Tests were further conducted on the adenosine-5'-chloracetophosphonate (III) on acetyl-CoA-synthetase removed from the heart of a rabbit. During this process it was found that in the presence of the given compound one observes a significant decrease in the rate of formation of the acetyl-coenzyme A. It was also established that the affinity of III ($K_i = 8 \cdot 10^{-6}$M) for the enzyme was noticeably higher than in the corresponding acetophosphonate ester of adenosine ($K_i = 1 \cdot 10^{-5}$M)[3].

For electrophoretic separation, we used the system 0.1 M acetic acid — 0.1 M ammonium acetate, pH 5.4 (system 1). Preparative separation was carried out on Whatman's Nr. 3MM chromatographic paper at a voltage gradient of 60 v/cm for a period of two hours. Chromatographic separation (descending chromatography) was carried out on Whatman's Nr. 1 paper, using the system ethanol-1 M ammonium acetate (7:3), pH 4.5 (system II). The derivatives of adenosine-5'--phosphate were determined according to absorption in UV light.

The diethyl ester of chloracetophosphonic acid. Of 62.1 g (0.45 mole) diethylphosphoric acid [10] and 10.35 g (0.45 mole) metallic sodium, a solution of sodium diethylphosphate in 1 l absolute ester is prepared. The obtained solution is added to a solution of 56 g (0.5 mole) chloracetylchloride in 75 ml absolute ester during stirring and cooling to -12° to -15°. The mixture is kept for 1.5 hours at 20°, and after removing the precipitate and distilling off the ester, the residual is distilled in a vacuum. Yield is 10.6 g (11%). Boiling point 116-118°/0.5 mm; n_D^{20} 1.4540.

Dichloranhydride of chloracetophosphoric acid. To 9.2 g (0.042 mole) diethyl ester chloracetophosphonic acid several portions 17.9 g (0.086 mole) dry phosphorous pentachloride are added. The reaction mixture is stirred for

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[3]The authors expressed their deep gratitude to Ye. V. Sharkova for the enzymatic test of the obtained compound and for determining the inhibition constant.

45 minutes at 80°, and it is then distilled in a vacuum. Yield is 3.2 g (55%). Boiling point 108-110°/1 mm; n_D^{20} 1.5000.

Adenosine-5'-chloracetophosphonate (III). To a solution of 0.6 g (0.002 mole), 2', 3'-0-isopropylidenadenosine (I) [11] in 21 ml absolute dioxane containing 0.48 g (0.006 mole) absolute pyridine are added 1.12 g (0.005 mole) chloranhydride of chloracetophosphonic acid while stirring and cooling to 12°. After 1 hour (20°), the precipitate is removed and dissolved in 7 ml H_2O and the pH of the solution is brought to ~6 with 5% aqueous ammonia. The solution is boiled dry at 30-35° and the residue is dissolved in 60 ml 0.1 N H_2SO_4 and left for 30 hours at 20°. The solution is neutralized by adding Dowex-1 /337 (OH^--form) resin to pH ~ 2 and is boiled dry. The residue is purified by preparative electrophoresis. After drying, strips of paper are cut out which have absorption in UV light with mobility of 0.8 of the mobility of adenosine--5'-monophosphate. Elution is carried out with water. III yield (in the form of ammonia salt) is 10-15% after purification, taking into account the initial product I. R_f 0.41 (system II); E_{amf} = 0.8 (system I)[4].

found %: C 32.28; H 4.64; Cl 7.93; P 7.21

$C_{12}H_{18}N_6O_7ClP \cdot H_2O$. calculated %: C 32.55; H 4.55; Cl 7:78; P = 6.99

[4] E_{amf} - electrophoretic mobility relative to adenosine-5'-monophosphate.

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Translated for the National Aeronautics and Space Administration under Contract No. NASw-2485 by Techtran Corporation, P.O. Box 729, Glen Burnie, Maryland, 21061; translator, Samuel D. Blalock, Jr.